REMARKS

Applicant apologizes for failing to adequately comply with 37 CFR § 1.821-1.825. In preparing a new CRF diskette to add the nucleotide and amino acid sequences pointed out in the Office Action mailed on 26 September 2001, Applicant's attorney inadvertently neglected to insert the amended paragraphs of the Specification into the Amendment text. That oversight has now been corrected.

You are hereby authorized to charge any fees due and refund any surplus fees to our Deposit Account No. 50-1796, referencing docket number 13054.02000.

Respectfully submitted,

CROSBY, HEAFEY, ROACH & MAY

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Fig. 10B shows an alignment of the amino acid sequences of *Arabidopsis* CKB1, CKB2, and CKB3. The amino acid identities between CKB3 and CKB1, CKB3 and CKB2, and CKB1 and CKB2 are 75%, 71% and 80%, respectively. The similarity is greatest over the carboxyl-terminal two thirds of the three proteins. The CKB3 protein shares most of the structural features of CKB1 and CKB2 at the level of primary structure (10). First, CKB3 contains a potential metal-binding motif Cys-Pro-X_3-Cys-X_{22}-Cys-Pro-X-Cys (SEQ I.D. No. 3) (45) (wherein "X" may be any amino acid). Second, although the conserved autophosphorylation site, Ser-Ser-Ser-Glu-Glu (SEQ I.D. No. 4), is missing in the amino-terminal region of CKB3, there are two CK2 recognition phosphorylation sites, ^{81}Ser-Gly-Ser-Glu-Gly-Asp (SEQ I.D. No. 5) and ^{83}Ser-Glu-Gly-Asp-Asp, (SEQ I.D. No. 6) in about the same location as in the animal β-subunits. Third, CKB3 has an N-terminal extension preceding the putative phosphorylation sites which exhibits a moderate level of similarity to the N-terminal extension of the other *Arabidopsis* β-subunits. Neither yeast nor animal β-subunits contain such an N-terminal extension, and this region bears no extensive similarity to other proteins.

Please replace the three paragraphs starting at line 10, page 19, with the following text:

Overexpression of *CKB3* To further explore the hypothesis that the CK2-CCA1 DNA-protein complex plays a role in the regulation of the circadian clock, we created transgenic *Arabidopsis* plants overexpressing a c-myc tagged form of *CKB3* and analyzed their circadian behavior. To produce the tagged *CKB3 an* Eco RI-Bsr GI fragment of the plasmid pUC-CKB3 that contains the entire coding sequence of *CKB3* cDNA at the Bam HI site of pUC19 was replaced with the duplex DNA composed of oligonucleotide myc-CKB3F (5'- AATTGAGATCTCATGGAGCAAAAGCTTATCAGCGAGGAGGACTT GAACAT) (SEQ I.D. No. 7) and oligo-nucleotide myc-CKB3B (5'-

GTACATGTTCAAGTCCTCCTCGCTGATAAGCTTTTGCTCCATGAGATCT) (SEQ I.D. No. 8) to introduce the Bgl II site and c-myc encoding sequence in front of *CKB3*. The resultant plasmid was digested with Bgl II and Hinc II, and the Bgl II-Hinc II fragment was subcloned into the pBI121 vector (Clonetech). This construct was used to transform *Agrobacterium tumefaciens* strain A2260, and then *Arabidopsis* plants (Columbia ecotype) using the *in planta* transformation procedure as described (61, 62). Overexpression of *CKB3* had no apparent effects on plant growth and development except timing of flowering.

From 16 transgenic lines that each had a single site of insertion, two transgenic lines designated ox18 and ox41 were further analyzed. Levels of *CKB3* transcript in the fourth generation of homozygous *CKB3*-overexpressing (*CKB3*-ox) plants were approximately 20 times higher than that in the wild-type (Fig. 10A). Ten μg of total RNA were treated with RQ1 RNase-free DNase (Promega) and the first-strand cDNA was synthesized as described in (43) The product of the first-strand synthesis was then used for PCR to amplify 140 bp *CKB3* cDNA with the primers CKB3F1 (5'-ACAAGGAACGTAGTGGAGGAGGTG) (SEQ I.D. No. 9) and CKB3B3 (5'-AACCCTAGATGT GGTGGTGGAAG) (SEQ I.D. No. 10). As a control, primers UBQ10-5' and UBQ10-3' (61, 62) were used to amplify 111 bp *UBQ10* cDNA. The resultant PCR fragments were separated on a 2% agarose gel, blotted and hybridized with ^{32}P-labeled probes.

The transgenic plants contained appreciable amounts of the c-myc-tagged CKB3 protein (Fig. 10B). Protein extracts were obtained by grinding 10-day-old seedlings in 100 μl of 3XSDS-sample buffer (180 mM Tris-HCl (pH 6.8), 6% SDS, 30% glycerol, 7.5% 2-mercaptoethanol), boiling this mixture for 5 min and saving the supernatant after centrifugation for 15 min at 14,000x*g*. Protein concentration was measured with a protein assay reagent (Bio-Rad). Western blots were performed using anti-c-myc monoclonal antibody 9E10 (17, 15, 64, 46) following the methods described in (61, 62).Measurement of CK2 activity in whole-cell extracts showed that the transgenic lines exhibited a 1.7-fold increase in CK2 activity (Fig. 10C). Frozen seedlings (100 mg) were ground and

extracted with 100 μl of extraction buffer (50 mM Tris-HCl (pH 7.5), 15 mM $MgCl_2$, 0.1 M KCl, 0.25 M sucrose and 10% glycerol, 1 mM phenylmethylsulfonylfluoride, protease inhibitor cocktail (Boehringer Mannheim), phosphatase inhibitor mixture (55, 26, 48) and 14 mM 2-mercaptoethanol). After centrifugation at 14,000x*g* for 15 min, the supernatant was saved and protein concentration was measur ed as above. CK2 assays were carried out at 37 °C with 200 μM CK2 specific peptide substrate Arg-Arg-Arg-Asp-Asp-Asp-Ser-Asp-Asp-Asp (SEQ I.D. No. 11) (Boehringer Mannheim) in 25 μl of CK2 buffer (55, 26, 48) as described (5).